CHAPTER 5

Variability of Protistan and Bacterial Communities in two Arctic fjords (Spitsbergen)

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Abstract

Krossfjorden and Kongsfjorden are Arctic fjords on the Western side of Spitsbergen. These fjords share a common mouth to the open sea and are both influenced by the inflow of warm transformed Atlantic waters as well as the input of sediment-rich glacial meltwater. The latter leads to decreased surface salinity, increased turbidity and decreased light penetration during summer. Earlier classical taxonomic studies had described the pelagic protistan composition of the Kongsfjorden during summer, revealing the dominance of flagellates of often unresolved taxonomic origin. Virtually no information existed on microbial eukaryote composition of the Krossfjorden as well as the bacterial composition of both fjords. The aim of the present study was to analyze and compare surface summertime protistan and bacterial communities in both fjords. Samples were collected three times a week from the central Kongsfjorden over a one month period. Additionally, 10 marine and 2 freshwater sites were sampled within a one week period in both Kongsfjorden and Krossfjorden. The microbial community composition was analyzed using molecular approaches (16S and 18S rRNA DGGE, sequencing). The central Kongsfjorden revealed a relatively stable protistan community over time with dinoflagellates, chlorophytes and small heterotrophs dominating. In contrast, the bacterial community varied and appeared to be correlated with the inflow of glacial meltwater. Additionally, the Kongsfjorden and Krossfjorden were found to harbor distinctive bacterial and eukaryotic communities. We speculate that differences in glacial meltwater composition and fjord bathymetry affect the surface water properties and therefore the observed spatial variability in the community fingerprints.

INTRODUCTION

Global climate change models predict enhanced glacial retreat and meltwater input, in polar coastal regions. Hop *et al*. (2006) suggested that the Arctic-Atlantic boundary location of the Kongsfjorden and Krossfjorden (Spitsbergen) places this system in a good position as an indicator for climate related changes. These semi-open glacial fjords share a common mouth to the open sea on the Western coast of Spitsbergen. Both are influenced by meltwater of glacial origin as well as by mild temperatures mediated by the inflow of transformed Atlantic water. The Krossfjorden and Kongsfjorden are glacial fjords fed with freshwater by respectively three and four major glaciers and by several streams originating from melting snow fields and glaciers located at higher elevations (Svendsen *et al.* 2002; Cottier *et al.* 2005). In the well studied Kongsfjorden, freshwater influx is highest in summer and co-occurs with a strong increase in sediment particle concentrations (Somerfield *et al.* 2006), which can reach up to 1000 $mg.dm^{-3}$ at the glacier front (Zajaczkowski 2002). The Kongsbreen glacier sediment discharge, measured as an inverse value of light beam attenuation, appears to be responsible for the strongest sediment input in the Kongsfjorden (Keck *et al.* 2001; Somerfield *et al.* 2006). Additionally, the meltwater discharge affects the salinity of surface waters up to 45 km from the glacier front and up to 30 m depth (Keck *et al.* 2001; Svendsen *et al.* 2002; Hop *et al.* 2006). Hanelt *et al*. (2004) measured Kongsfjorden surface water salinity values varying from 24 to the normal 34 psu throughout summer.

The Kongsfjorden and Krossfjorden are also strongly influenced by the West Spitsbergen Current (WSC) of Atlantic origin that transports relatively warm saline water (psu > 34.7) northwards (Falk-Peterson *et al.* 2000; Keck *et al.* 2001; Svendsen *et al.* 2002; Drinkwater 2006; Schlichtholz and Goszczko 2006; Hop *et al.* 2006). As a result, both fjords are characterized by relatively mild temperatures as compared to other Arctic locations at similar latitude. Average water temperatures slightly above 0 ^{o}C prevent substantial sea ice formation in winter, which is limited to the edges and inner parts of the fjords, whereas the central and outer parts remain ice-free throughout most winters (Svendsen *et al.* 2002).

Mixing of the warm WSC water with glacial freshwater and enhanced sediment concentration are important determinants for phytoplankton growth and species composition of West Spitsbergen fjords. High sediment concentrations significantly limit light penetration into the water column, which can reduce the euphotic zone to 0.3 m (Keck *et al.* 2001) leading to unfavorable conditions for phytoplankton growth (Hop *et al.* 2006). While, events of transformed Altantic water inflow can affect the phytoplankton community by transporting non-Arctic species into the fjords, like coccolithophorid species as reported by Hasle and Heimdal (1998). Strong relationship between water mass advection and changes in zooplankton community structure have already been observed in the Kongsfjorden (Willis *et al.* 2006).

Only a limited number of studies have been devoted to the characterization of Arctic marine microbial eukaryotic and prokaryotic communities in general (Bano and Hollibaugh 2002; Lovejoy *et al.* 2002; Lovejoy *et al.* 2006; Malmstrom *et al.* 2007; Galand *et al.* 2008). Yet, the predicted and already observed effects of Arctic climate change require a comprehensive insight in present-day microbial community composition, as well as a better

understanding of the environmental factors that determine their *in situ* performance. Classical taxonomic studies conducted on protistan diversity of the Kongsfjorden revealed a spring bloom dominated by diatoms and *Phaeocystis pouchetii*, followed by a more diverse summer community. Up to 134 taxa have been reported from microscopic surveys of the pelagic community from Kongsfjorden (Hasle and Heimdal 1998; Keck *et al.* 2001; Hop *et al.* 2002). Unfortunately, many representatives of the protistan community are difficult to determine using classical microscopy: the cryptic morphologic features of the often tiny flagellates render their identification by microscopy extremely challenging, even for the more experienced taxonomist. With respect to the Krossfjorden, virtually no information was available on protistan abundance or composition.

Molecular techniques offer an efficient, high resolution approach with added value to classical micro-eukaryotic community analysis, since molecular species recognition may go beyond microscopic resolution. This would apply to the summertime communities of Kongs- and Krossfjorden in particular. Moreover, no data have yet been published on pelagic bacterial communities from the Kongsfjorden and Krossfjorden, in contrast to other Arctic sites (Bano and Hollibaugh 2002; Lovejoy *et al.* 2002; Lovejoy *et al.* 2006; Malmstrom *et al.* 2007; Galand *et al.* 2008). Finally, simultaneous characterization of the Kongsfjorden-Krossfjorden surface protistan and bacterial communities was missing.

The aim of the present study was to analyze surface summertime protistan and bacterial communities as a function of environmental properties. We investigated changes in microbial communities over a one-month period in summer for one particular location in the Kongsfjorden. We also studied spatial differences among several sampling locations in both Kongs- and Krossfjorden. The microbial community composition was analyzed using 16S and 18S rRNA community fingerprinting and sequencing. Additionally we explored which environmental variables correlated most strongly with the observed differences in community composition for both the temporal and the spatial study.

MATERIAL AND METHODS

Sampling strategy

Surface samples were collected weekly from a fixed location (78°55.813'N 11°56.674'E, TS, Fig.5.1) in the Kongsfjorden 200 m north off the coast of Ny-Ålesund from June 8th until July 8th, 2005. Additionally, 10 marine stations and 2 freshwater stations were sampled to compare microbial communities between fjords. As a strongly reduced surface salinity in the fjords might benefit organisms originating from freshwater sources we also characterized freshwater communities. These samples were collected within one week (June 25th to July 2nd). Samples F1-F4 were taken in the Krossfjorden; F6-F8, BF (Bayelva Fjord), DD (Drinking-water Delta) in the Kongsfjorden and sample F5 was taken from a location at the intersection of the Kongsfjorden and Krossfjorden (Fig. 5.1). Freshwater was sampled from the Bayelva River (B) and the Drinking-water Lake (D), both streaming into the Kongsfjorden (Fig. 5.1). Each sample was pre-filtered over a 200 µm mesh and subsequently 1.5 L was filter fractionated by vacuum pressure onto polycarbonate filters of 10 µm, 2 µm and 0.2 µm pore size (Millipore).

This yielded three size fractions: the 200-10 µm, 10-2 µm and 2-0.2 µm fractions, henceforth named after their pore size. The filters were stored in 2 mL screw-cap tubes at -80°C until further processing.

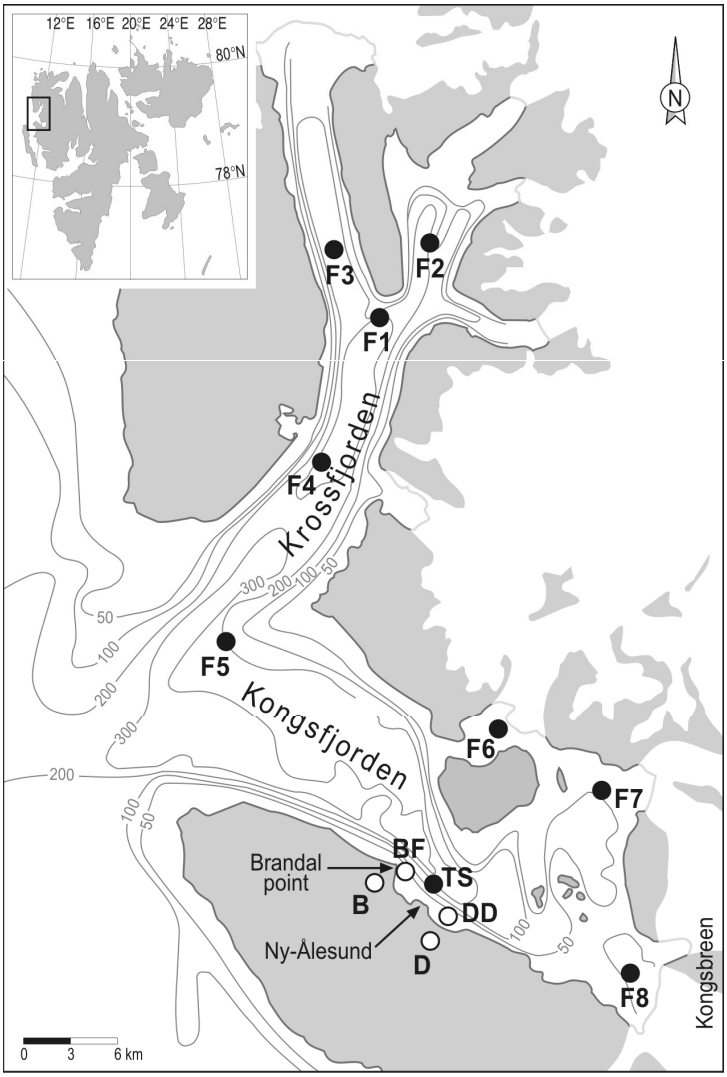


Figure 5.1. Bathymetric map of Kongsfjorden and Krossfjorden (77°30 to 78°55'N and 11°30 to 11°56'E) with sampling locations.

Samples F1 to F4 were collected in the Krossfjorden; F6–F8, TS, BF and DD samples collected in the Kongsfjorden; F5 at the intersection of both fjords. B and D represent freshwater samples, taken respectively from the Bayelva River and the Drinkwater Lake. TS: "temporal station" used for the 5.5 weeks survey.

Molecular analysis

All filter-handling steps were performed under sterile conditions. Filters were cut into small pieces, transferred to screw-cap tubes containing 1 mL of lysis buffer (EDTA 40 mmol.L^{-1}; Tris-HCl 50 mmol.L^{-1}, pH 8.5; sucrose 0.75 mol.L^{-1}), thoroughly vortexed and incubated for one hour at room temperature. Given the high sediment content in several samples, DNA was extracted with the MoBio UltraClean™ Soil DNA Kit following the manufacturer's protocol for wet soil samples applying the alternative lysis method. 18S rRNA gene fragments of approximately 500bp were amplified using the eukaryote-specific primer set Euk1A and 516r-GC as described by Díez *et al.* (2001). Primer 516r-GC contains a GC-clamp at the 5'-end for Denaturing Gradient Gel Electrophoresis (DGGE) application (Muyzer *et al.* 1993; Nübel *et al.* 1996). A fragment (~430bp) from the variable V6 region of bacterial 16S rRNA gene was amplified using the U968f-GC forward primer, containing a GC clamp at the 5'end, and the U1401R reverse primer (Nübel *et al.* 1996). The 18S rRNA polymerase chain reaction (PCR) mixture (20 µL final volume) consisted of 1x PCR buffer (Amersham), 3.25mmol.L^{-1} $MgCl_2$, 1% formamide, 0.2 mg.mL^{-1} Bovine Serum Albumine (Roche), 200 µmol.L^{-1} dNTP's, 300 nmol.L^{-1} Euk1A, 300 nmol.L^{-1} 516r-GC (Eurogentec) and 1U Taq-DNA polymerase (Amersham) in Ambion Ultra-clean water. For the 16S rRNA PCR reaction the concentrations were identical except for the primers added in 200 nmol.L^{-1} final concentration. The 18S rRNA amplification cycle was performed as described in Díez *et al.* (2001). The 16S rRNA PCR started with a 5 min 94 ^{o}C denaturation step, followed by 10 touchdown cycles with annealing temperatures decreasing 0.5 ^{o}C each cycle from 60 to 55 ^{o}C, and then 25 cycles of 60 s at 94^{o}C, 60 s at 55 ^{o}C and 120 s at 72 ^{o}C. The amplification was finalized by an additional step at 72 ^{o}C for 30 min to remove artificial double bands (Janse *et al.* 2004). Amplification products were analyzed by electrophoresis on a 1% (w/v) agarose gel.

DGGE

PCR reactions producing bands of the proper size and quantity were subjected to DGGE analysis using the PhorU system (Ingeny). Optimal separation for the 18S rRNA fragments was obtained with a 10-60% Urea-Formamide gradient and a 40-70% gradient for the 16S rRNA fragments. For each sample 200 ng of PCR product was loaded with 1x loading buffer (0.05% w/v bromophenol blue, 40% w/v sucrose, 0.1 mol.L^{-1} EDTA pH 8.0, 0.5% sodium lauryl sulfate). Analysis of the DGGE patterns was performed using BioNumerics® version 3.5 (Applied Maths). Gel images were digitalized and normalized with flanking marker samples. Automated band calling was performed in BioNumerics using standard detection settings (manufacturer's protocol); however with a few precautions as stains in the gel may lead to false positives and bands with low intensities may remain undetected. Consequently, a visual check of the obtained band patterns was performed in order to remove false positives and add low-intensity bands. In BioNumerics, band patterns were translated into a presence and absence matrix, and relative band intensity was inferred from densitometric curves. A matrix of distances between densitometric curves generated from the fingerprint was calculated as Pearson's product-moment correlation coefficients between patterns, using the unweighted pair group method with arithmetic averages (UPGMA). Similarities in fingerprints were presented in the form of a dendrogram.

Sequencing

A subset of the samples was chosen for cloning and sequencing such that all bands detected in the environmental fingerprints were included. Clone libraries of 18S rRNA fragments (position 1-516) were generated from 8 samples: F5-2, BF-10, D-10, D-2, D-0.2, B-10, B-2, B-0.2 (location name – size fraction); and for the 16S rRNA (*Escherichia coli* position: 968-1401) from four samples: F4-2, F8-10, D-2 and B-2. DNA fragments were cloned in pGEM-t vector (Promega) and transformed to *E. coli* strain JM109 according to the manufacturer's protocol. Positive inserts were selected and DNA was amplified using the DGGE primer sets and re-run on a DGGE next to the environmental sample from which the clone library was made. Migration patterns of PCR products from these single clones were compared to the original environmental band patterns. Only clones that gave DGGE-PCR products corresponding to bands in the environmental pattern were selected for subsequent sequencing. The 18S rRNA clone libraries generated from the contrasting locations F5 and BF proved sufficient to generate all the bands detected in marine samples from the Kongsfjorden and Krossfjorden, whereas the freshwater samples (B and DL) required more samples to generate clones representing all the bands observed in the freshwater fingerprints. The pGEM-t inserts were amplified from the vector's T7-SP6 sites followed by a cleanup by polyethylene glycol 8000 (Sigma) precipitation. Twenty µL sequence reactions were performed with Big Dye buffers and 200 $nmol.L^{-1}$ primer: T7 primer for the 16S rRNA inserts, and Euk1A primer for 18S rRNA inserts. After an initial denaturation step, the sequence program consisted of 25 cycles of 10 s at 96 ^{o}C, 5 s at 50 ^{o}C and 4 min at 60 ^{o}C with a final hold at 4 ^{o}C. The Products were cleaned by standard isopropanol precipitation and analyzed on an automated ABI 377 DNA sequencer (Applied Biosystems).

Sequence data were manually checked with Chromas v.2.3.1 and suspected chimeric sequences were checked online using the Ribosomal Database Project II 8.1 Chimera Check program (http://rdp8.cme.msu.edu/cgis/chimera.cgi?su=SSU). For each sequence, the closest match of a cultivated strain obtained with NCBI BLAST was included in the phylogenetic analysis (http://www.ncbi.nlm.nih.gov/BLAST). Molecular Evolutionary Genetics Analysis (MEGA) version 4.0 (Tamura *et al.* 2007) and its add-in *ClustalW* was used to align the DNA sequences and to create neighbour joining trees based on the maximum composite likelihood (Hartl *et al.* 1994; Zhu and Bustamante 2005) algorithm with 1000 bootstrap permutations (Felsenstein 1985). Operational Taxonomic Units (OTUs) were defined as sequences with at least 97% identity. In the phylogenetic tree, OTUs were classified at the taxonomic phylum and class level as deduced from sequences of known taxonomy added from the NCBI database.

Environmental variables

Solar radiation and wind speed were continuously measured by the NDSC project (Koldewey Station, AWI, Potsdam, Germany). The wind speed was measured in $m.s^{-1}$ in an open terrain at 10 m height near Ny-Ålesund. The average wind speed measured on sampling days and average wind direction of the 7 days preceding each sampling day were taken for our analysis. Photosynthetically active radiation (PAR: 400-700 nm) and ultraviolet radiation (UV-R: 280-400 nm) were measured in $W.m^{-2}$ and recorded per minute. The mean incident daily irradiance was averaged over the days preceding each sampling day (7 days). Salinity was measured in

Lugol-preserved surface samples (Buma *et al.* 2001). From each water sample (< 200 µm) 250 mL was fixed with 2 mL Lugol and stored cool (4°C). In Buma *et al*. (2001) Lugol fixation had been shown not to significantly affect salinity measurements: variability with non-fixed samples was <0.1 psu, which was considered acceptable for a rough estimate of freshwater input in the present study. Although we did not measure sediment content directly, visual observation was used to provide indicative indices for the sediment content of surface water ranging from 2 (turbid, brown/red coloration), 1 (intermediate turbidity) and 0 (clear, deep-blue coloration).

Data analysis

The correlation between the relative abundance of prokaryotic and eukaryotic bands, and the environmental variables from location TS was estimated by calculating Spearman rank-order correlation coefficients using the software package SPSS statistics 14.0. Shannon-Wiener's diversity index of the cloned samples was calculated with the PAST software (PAlaeontological STatistics analysis program, http://folk.uio.no/ohammer/past/).

Ordination was performed on the absence and presence data of 62 bands of the 16S and 18S rRNA DGGE (including several unidentified bands) of ten marine samples: four from the Krossfjorden (F1-F4), five from Kongsfjorden (F6-F8, DD and BF) and the intersection location (F5); and on the values of the environmental variables *location* (Kongs- versus Krossfjorden) and *salinity*. We excluded the freshwater samples from this analysis. Principal component analysis (PCA), unimodal detrended correspondence analysis (DCA) and redundancy analysis (RDA) (Van den Wollenberg 2007) calculations were performed with CANOCO version 4.5.2 (Ter Braak and Šmilauer 1998). PCA is an indirect method of ordination that plots species according to the largest variation within the multivariate dataset on the x-axis, and the second largest variation (constrained by the first axis) on the y-axis. In this way, the relative species locations on the plot indicate strength of similarity according to the two most explanatory dimensions of the multivariate dataset. The environmental variables that make up this multivariate dataset were added later to the plot in order to see how species and environmental variables correlate. DCA and RDA can directly test for effects of environmental variables on the species data.

Nucleotide sequence accession numbers

All sequences presented in this study were submitted to NCBI GenBank database under accession numbers: **EU078179** to **EU078273**.

RESULTS

Environmental data

Analysis of the environmental variables (Table 5.1) revealed that salinity was highly variable over time and for locations within and between the fjords. Krossfjorden surface salinities were on average lower than in the Kongsfjorden; the inner fjord locations F1, F2 and F8 from both fjords had salinities < 20 psu. At location TS, the surface salinity decreased over time: with a strong salinity decrease in weeks 5 and 5.5. Simultaneously, in weeks 4, 5 and 5.5 sun hours and solar irradiation were high; following a period of very overcast weather (week 3). From week 3 onwards we observed a clear increase in sediment discharge into the Kongsfjorden: a brown/red plume of sediment-enriched water could be observed in the entire eastern side of the fjord up to Brandal Point (Fig. 5.1).

Table 5.1. Environmental variables available for station TS (Time) and for Kongs-Krossfjorden sampling stations (Location).

	Time					
	Week 1	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 5.5**
salinity (psu)	27.3	33.3	30.5	29.2	20.9	22.7
sediment index	0	0	0	2	2	1
~sun hours ($h.d^{-1}$)	9.65	7.18	0.7	12.22	10.05	14.68
~solar radiation ($W.m^{-2}$)	236.98	222.68	154.53	265.11	230.56	273
UV radiation ($W.m^{-2}$)	12.35	11.63	8.77	12.22	10.8	11.69
~wind speed ($m.s^{-1}$)	1.04	2.64	3.37	2.65	2.41	3.11
~wind direction (0 - 360°)	241.57	220.86	193.14	184.31	172.54	249.76
wind direction (0 - 360°)	249.18	153.84	268.74	147.22	125.15	207.07

	Location									
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**B**	**D**
salinity (psu)	17,8	16,3	22,6	21,6	25,1	28,1	23,3	19,6	23,3	17,5
sediment Index	0	0	0	0	0	1	2	2	2	1

Data with a '~' are average values calculated for the 7 days preceding sampling; other values were measured on the actual sampling day.

Microbial community fingerprints from the mid-Kongsfjorden

The DGGE pattern obtained for the 18S rRNA samples collected in the mid-Kongsfjorden, at location TS, showed minor variation over time (Fig. 5.2a). Most DGGE bands were present in all samples regardless of the size fraction, except for one band only detected in the larger size fractions of samples taken from weeks 1 to 3. Only minor changes in relative abundance (band intensity) were observed over time. The dendrogram obtained for these samples showed a simple clustering (data not shown), where all samples had at least 93% similarity in banding pattern showing 2 main clusters with samples from week 4 and the 2 µm and 0.2 µm from week 5 clustering separately from the rest.

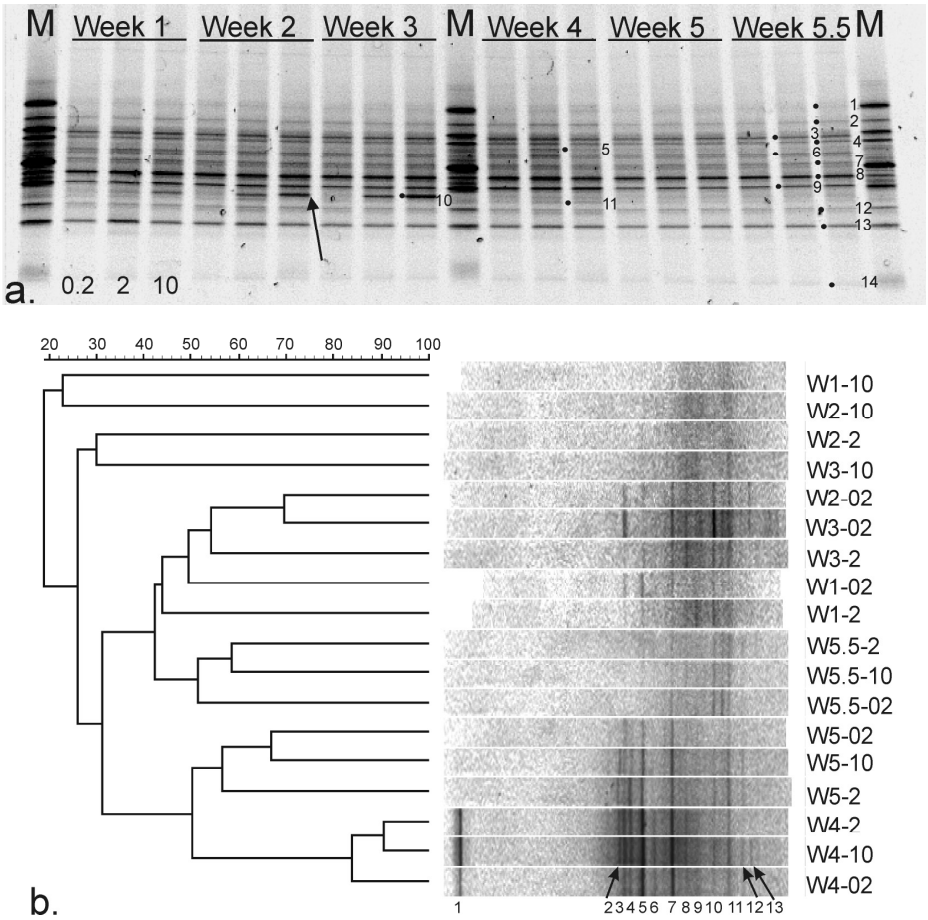


Figure 5.2. Fingerprints of surface marine microbial communities from the mid-Kongsfjorden location TS collected over a 5.5 weeks period.

a. Negative image DGGE gel of eukaryote 18S rDNA fragments from location TS collected from the 8th of June until the 8th of July. Each sample consists of three size fractions in the order: 0.2 µm, 2 µm and 10 µm. M, the marker sample, corresponds to sample DD-10 from the spatial study. A single band, indicated by the arrow was only present from week 1 to 3 in the larger size fractions and disappeared in the subsequent samples. Numbers indicate bands as determined by BioNumerics and are similar to those used in the correlation analysis (BE1 to BE14).

b. UPGMA dendrogram based on Pearson's similarity coefficient of densitometry curves obtained for the DGGE banding pattern of the bacterial community sampled over time. Sample annotation corresponds to weeks 1 to 5.5 (W1 to W5.5) followed by the corresponding size fraction (0.2, 2, or 10 µm), numbers indicate band numbers as determined by BioNumerics and are similar to those used in the correlation analysis (BP1 to BP13).

In contrast to the eukaryotic (18S rRNA) community, the bacterial community revealed strong variations in band patterns over the sampling period. In the first three weeks only the smallest size fraction revealed a distinct banding pattern, and accordingly the smaller size fractions of weeks 1, 2 and 3 clustered together (Fig. 5.2b). In weeks 4 and 5 the community changed and bands were also detected in the larger size fractions. Pearson's similarity correlation revealed a high similarity (>80%) for all size fractions of week 4. Samples of week 4 and 5 formed a separate cluster compared to the other weeks. On the last sampling day (week 5.5) the intensity and number of bands decreased together with a band pattern shift. The week 5.5 cluster showed more similarity to the cluster from samples of weeks 1 to 3.

DGGE band patterns analyzed by BioNumerics were translated into relative abundances. A total of 14 bands (BE1 to BE14; numbered in fig 5.2a) of eukaryotic origin and 13 bands (BP1 to BP13; fig. 5.2b) of bacterial origin were detected in the samples collected at location TS. Pairwise Spearman rank correlation analysis of the environmental variables and relative abundance of 16S and 18S rRNA DGGE bands showed several significant correlations (Table 5.2). The variable sediment load correlated significantly with nine eukaryotic and four bacterial bands. Seven eukaryotic bands showed a negative correlation to sediment index, while three out of four of the bacterial bands showed a positive correlation. Noteworthy, the variable wind direction was significantly correlated to the relative abundance of a few bacterial and eukaryotic bands.

Table 5.2. Spearman rank correlation coefficients between environmental variables and relative abundance of bands from the temporal DGGE generated for 16S (BP) and 18S (BE) rDNA fragments.

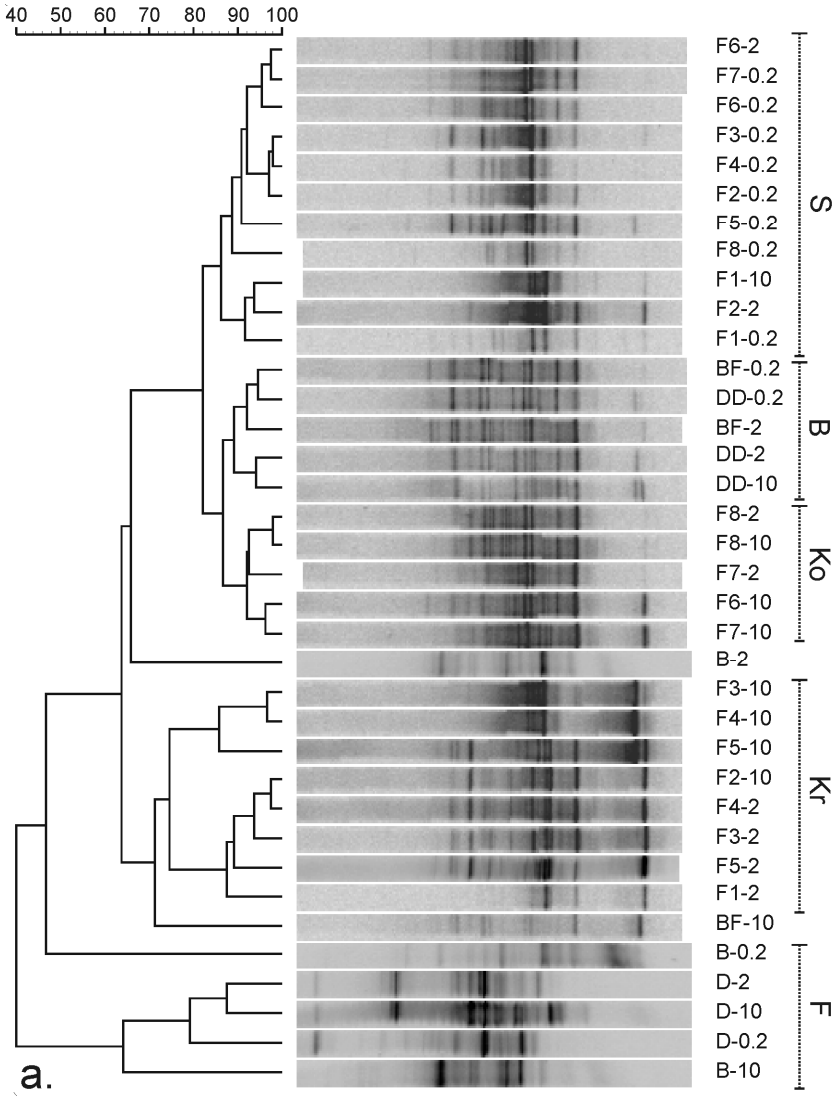
	BP2	BP3	BP4	BP5	BP6	BP8	BP9	BP10	BE1	BE2	BE3	BE4	BE5	BE6	BE8	BE9	BE10	BE12	BE13	BE14
Sun	***.83**	.37	.03	.37	.12	.77	-.64	-.76	.26	-.66	-.66	-.71	.44	-.77	-.6	-.6	.66	-.09	-.6	-.26
WS	.37	-.27	-.49	-.27	***-.87**	-.09	.4	-.21	-.43	-.2	-.2	.2	-.14	.14	-.09	-.31	.09	.03	-.31	-.26
~WD	-.03	***-.84**	***-.83**	***-.85**	-.52	-.43	-.15	.33	-.09	.54	.54	.49	-.78	.49	.6	.6	-.37	***.89**	.6	***.83**
WD	-.54	***-.85**	-.54	***-.85**	-.61	-.6	.15	.76	-.09	.54	.54	.66	-.78	***.89**	.6	.66	-.77	***.83**	.68	.6
Sal	-.37	-.51	-.37	-.51	-.46	-.66	****.94**	.46	***-.87**	.54	.54	***.83**	-.3	.43	.71	.37	-.6	.09	.37	.37
SED	.74	***.88**	.56	***.88**	.41	****.93**	-.42	***-.89**	.28	****-.93**	****-.93**	****-.93**	***.88**	****-.93**	****-.93**	****-.93**	***.83**	-.68	****-.93**	-.8

Abbreviations: **Sun**= Sun Hours, **WS**= wind speed on sampling day, **~WD**= average wind direction, **WD**= wind direction on sampling day, **Sal**= salinity, **SED**= observed sediment index, BP1-14 = Band Prokaryote, bands of the 16s DGGE and BE1-14 = Bands Eukaryote 1 to 14 from the temporal DGGE. NB. Bands lacking any significant correlation were removed from the table. Figures in grey are significant at the 0.05 (*) and 0.01 (**) level.

Kongsfjorden-Krossfjorden comparison

Cluster analysis of micro-eukaryotic community fingerprints generated for samples collected from different locations in the Kongsfjorden and Krossfjorden revealed a clustering according to size fraction and location (fig. 5.3a). Five clusters became apparent. The 0.2 µm size fraction of marine samples (denoted with S) grouped together, while the remaining samples revealed two different repetitive band patterns. One pattern within locations F1-F5 and another within locations F6-F8, BF and DD, matching the Krossfjorden (Kr) and Kongsfjorden (Ko+B) locations, respectively. Finally, band profiles for the freshwater samples D and B clustered together (F) and strongly differed from the seawater samples.

Cluster analysis of bacterial community fingerprints also unveiled clustering of samples mainly according to location and size fraction (Fig. 5.3b). Here, freshwater samples B and D formed a distinct cluster (F) separate from the Kongsfjorden and the Krossfjorden samples. Interestingly, the 16S rRNA band patterns obtained for the samples DD and BF were more similar to the Krossfjorden band patterns than the Kongsfjorden samples. Although the different size fractions appeared very similar within sampling locations in the DGGE analysis, band pattern analysis of the Krossfjorden revealed that the larger size fractions (Kr (10u)) clustered separately from the 0.2 µm size fraction (Kr (S)). The Kongsfjorden samples mainly clustered according to location.



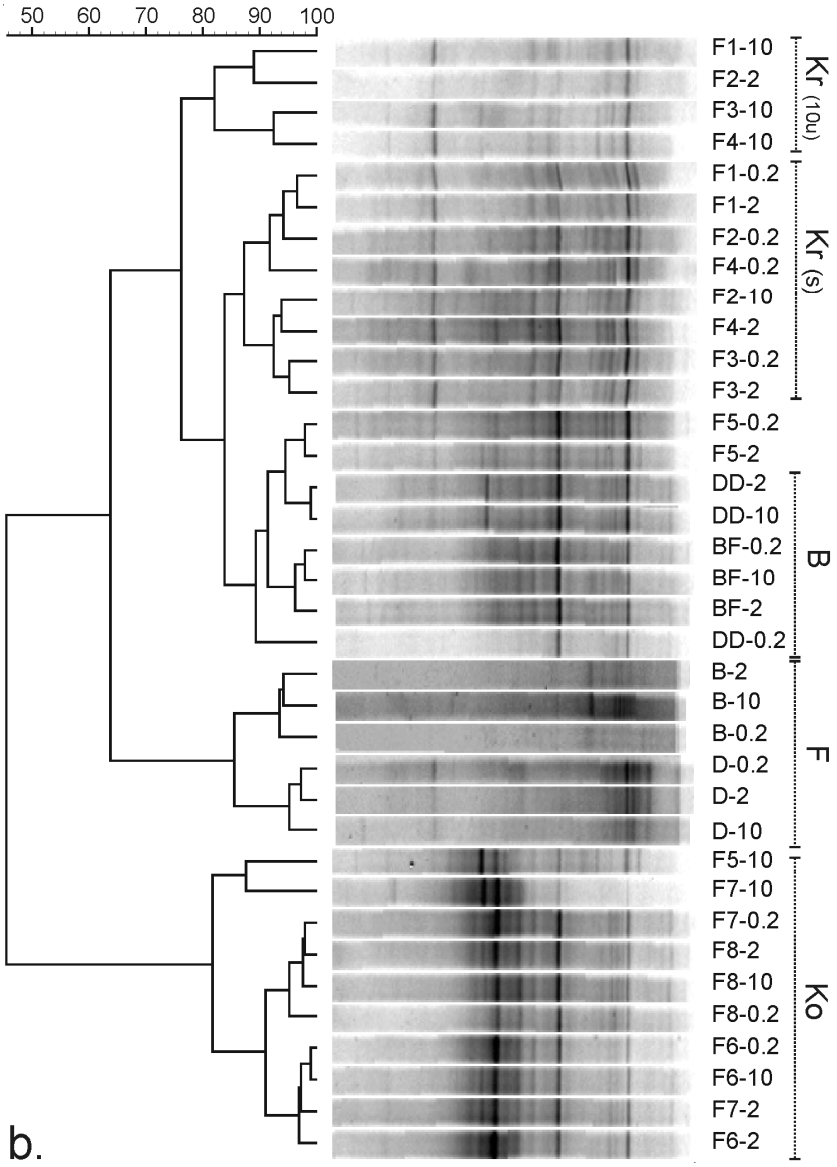


Figure 5.3. UMPGA dendrogram based on Pearson's similarity coefficient of densitometry curves obtained for (**a**) the eukaryotic 18S rDNA fragments and (**b**) bacterial 16S rDNA fragments from samples of the spatial study.(*pages 120&121*)

Sample codes correspond to the location followed by the fraction size. Main clusters were: F: Freshwater; B: outflow sites BF & DD; S: small size fraction; Ko: Kongsfjorden; Kr: Krossfjorden; Kr (10u): Krossfjorden large size fraction; Kr (S): Kr small size fraction.

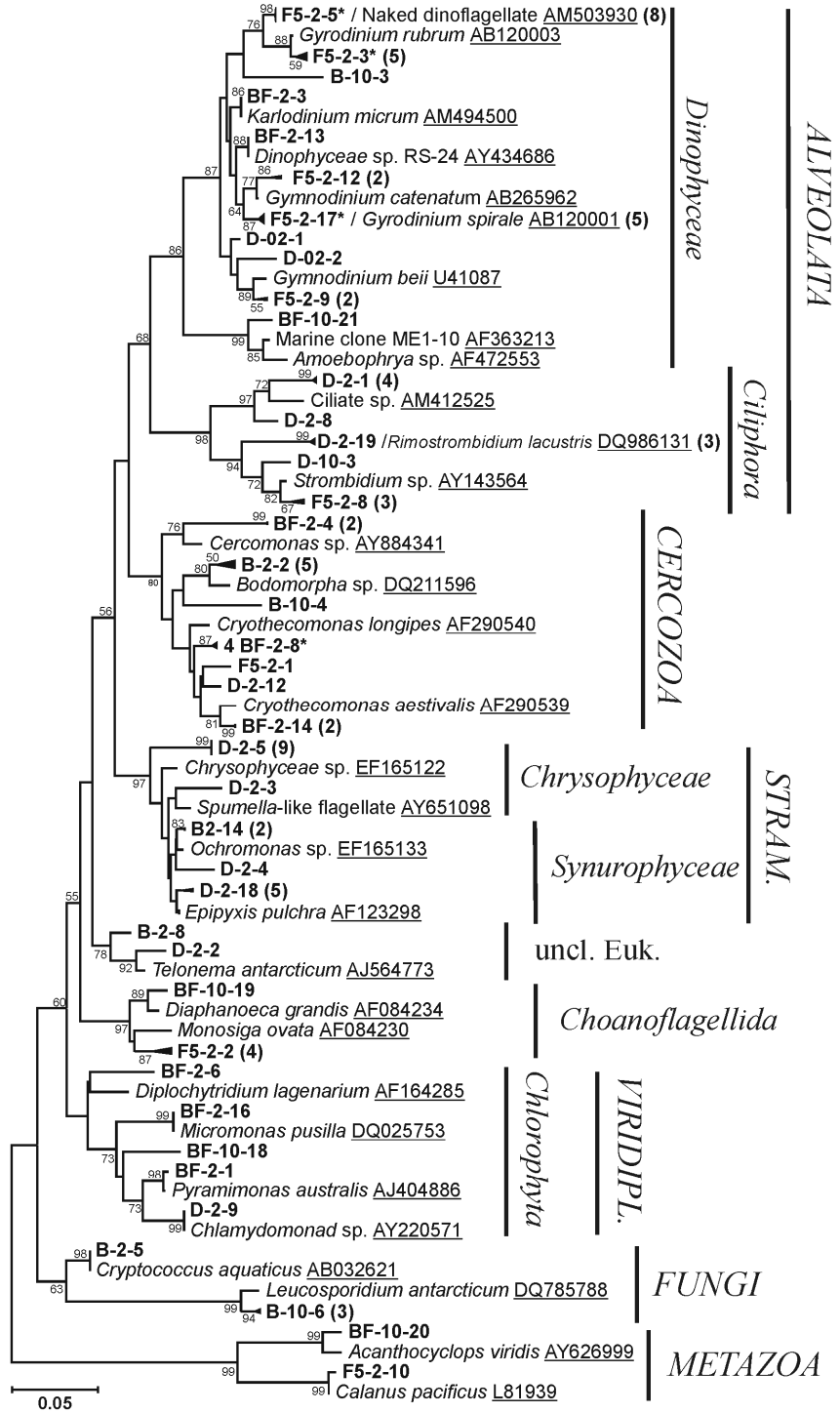
Ordination analysis

Detrended correspondence analysis (DCA) revealed that the length of gradients was lower than 2. Therefore, we used redundancy analysis (RDA) to perform ordination analysis on our data set. The environmental variables *location* and *salinity* significantly explained 25.7% of the species variation in the data set from the marine samples (Monte Carlo permutation test, p = 0.006). When the significance of the variables were tested separately, only the variable *location* explained a significant part of the variation (23.7%, p= 0.009). Accordingly, the PCA triplot revealed two main clusters: the Krossfjorden and Kongsfjorden samples, including the Bayelva fjord (BF) sample. The drinking-lake delta sample (DD) and F5 sample were positioned separately from the Kongs- and Krossfjorden samples.

Sequence data

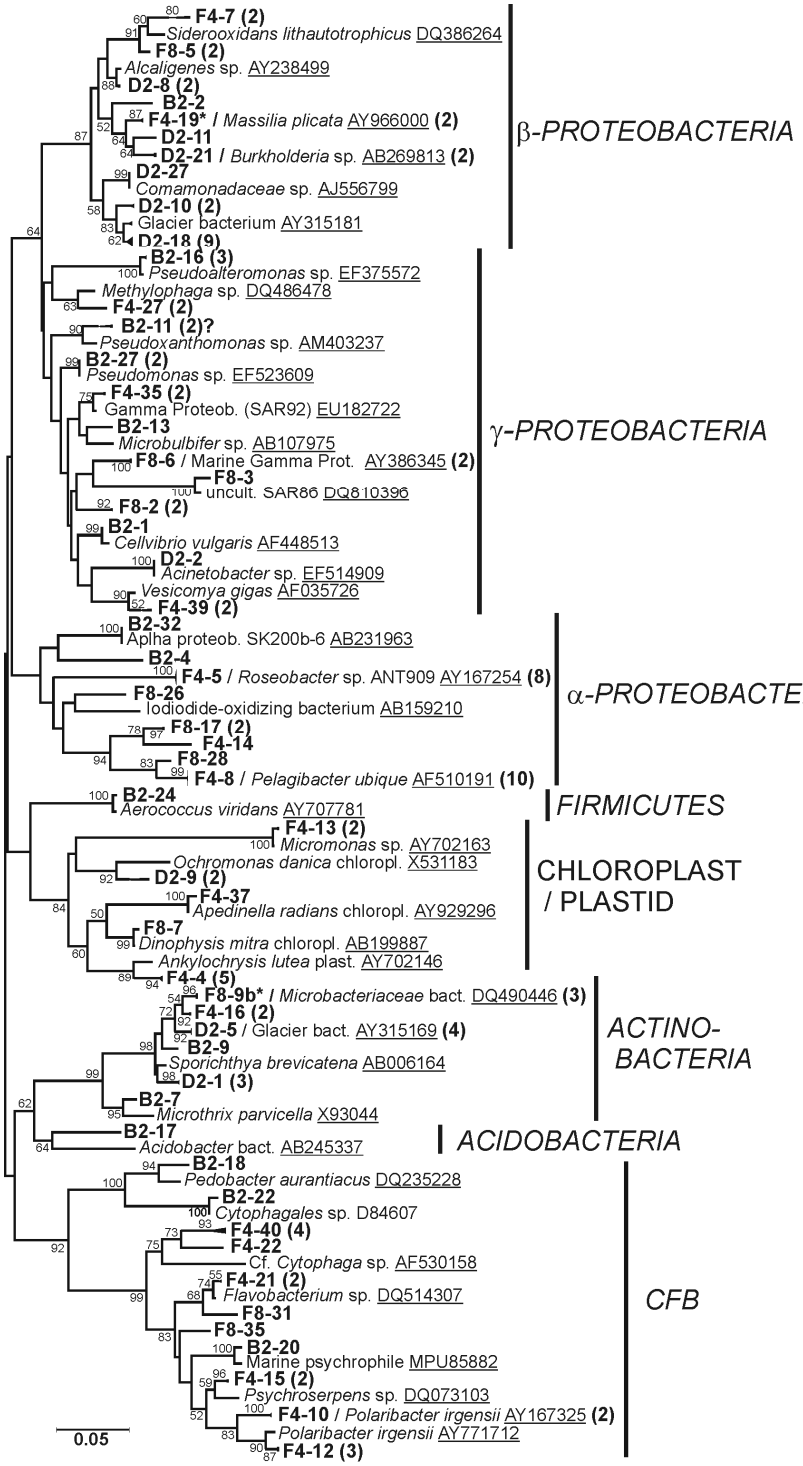
Sequences with >97% identity were assigned to the same operational taxonomic unit (OTU). Shannon-Wiener diversity index (H_0) of the cloned samples averaged 2.5 and 2.8 respectively for the 18S rRNA and the 16S rRNA diversity; we found a total of 41 OTUs out of 92 18S rRNA clones and 54 OTUs out of 115 16S rRNA clones. Phylogenetic classification of the eukaryote sequences (Fig. 5.4) revealed the presence of *Dinophyceae*, *Ciliphora*, *Cercozoa*, *Choanoflagellida*, *Chlorophyta* and *Copepoda* (grouped under *Metazoa*) in saltwater locations. OTUs related to *Chrysophyceae*, *Synurophyceae*, *Telonema* sp. and *Fungi* were only found in freshwater samples. A few OTUs related to the *Dinophyceae* (*Gyrodinium rubrum*, *Gyrodinium spirale*) and *Cercozoa* (*Cryothecomonas* sp.) were found in both fresh- and saltwater habitats. In our samples *Dinophyceae* were the most abundant and diverse group with 28 sequences divided over 11 OTUs.

Figure 5.4. Neighbor-joining phylogenetic tree showing the position of partial 18S rRNA sequences obtained from the generated clone libraries (*page 123*).
OTU code names correspond to: sampling location, fraction size, clone number. STRAM. stands for Stramenopiles; VIRIDIPL.: *Viridiplantae*; uncl. Euk.: unclassified *Eukaryota*. Numbers between brackets are number of clones clustering within the OTU. Bootstrap values > 50% are indicated in front of nodes. * indicate OTUs detected in both freshwater and marine samples.


F5-2-5* / Naked dinoflagellate AM503930 (8)
Gyrodinium rubrum AB120003
F5-2-3* (5)
B-10-3
BF-2-3
Karlodinium micrum AM494500
BF-2-13
Dinophyceae sp. RS-24 AY434686
F5-2-12 (2)
Gymnodinium catenatum AB265962
F5-2-17* / Gyrodinium spirale AB120001 (5)
D-02-1
D-02-2
Gymnodinium beii U41087
F5-2-9 (2)
BF-10-21
Marine clone ME1-10 AF363213
Amoebophrya sp. AF472553
Dinophyceae
D-2-1 (4)
Ciliate sp. AM412525
D-2-8
D-2-19 /Rimostrombidium lacustris DQ986131 (3)
D-10-3
Strombidium sp. AY143564
F5-2-8 (3)
Ciliphora
ALVEOLATA
BF-2-4 (2)
Cercomonas sp. AY884341
B-2-2 (5)
Bodomorpha sp. DQ211596
B-10-4
Cryothecomonas longipes AF290540
4 BF-2-8*
F5-2-1
D-2-12
Cryothecomonas aestivalis AF290539
BF-2-14 (2)
CERCOZOA
D-2-5 (9)
Chrysophyceae sp. EF165122
D-2-3
Spumella-like flagellate AY651098
Chrysophyceae
B2-14 (2)
Ochromonas sp. EF165133
D-2-4
D-2-18 (5)
Epipyxis pulchra AF123298
Synurophyceae
STRAM.
B-2-8
D-2-2
Telonema antarcticum AJ564773
uncl. Euk.
BF-10-19
Diaphanoeca grandis AF084234
Monosiga ovata AF084230
F5-2-2 (4)
Choanoflagellida
BF-2-6
Diplochytridium lagenarium AF164285
BF-2-16
Micromonas pusilla DQ025753
BF-10-18
BF-2-1
Pyramimonas australis AJ404886
D-2-9
Chlamydomonad sp. AY220571
Chlorophyta
VIRIDIPL.
B-2-5
Cryptococcus aquaticus AB032621
Leucosporidium antarcticum DQ785788
B-10-6 (3)
FUNGI
BF-10-20
Acanthocyclops viridis AY626999
F5-2-10
Calanus pacificus L81939
METAZOA
0.05

The 16S rRNA sequences revealed the presence of γ-, α-, β-*Proteobacteria*, *Actinobacteria* and *Cytophaga-Flavobacteria-Bacteroidetes* (grouped under CFB) in both marine and freshwater samples (Fig. 5.5), while sequences of bacteria belonging to the *Firmicutes* and *Acidobacteria* were confined to freshwater samples. Eleven sequences were related to chloroplasts and plastids, organelles of bacterial origin (Kuhsel *et al.* 1990), including sequences related to the *Ochromonas* sp. from freshwater samples and *Micromonas* sp. from marine samples that were also detected by 18S rRNA gene sequencing. A few clones of freshwater and marine samples clustered within the same OTU: F4-19 (β-*Proteobacteria*) and F8-9b (*Actinobacteria*). Overall, most classes had representatives in both habitats: the γ-*Proteobacteria* was most diverse with 12 OTUs, followed by the *Bacteroidetes* and β-*Proteobacteria* with 11 and 10 OTUs, respectively.

Figure 5.5. Neighbor-Joining phylogenetic tree showing the position of partial 16S rRNA sequences (*page 125*). For coding see legend of fig. 5.4.


F4-7 (2)
Siderooxidans lithautotrophicus DQ386264
F8-5 (2)
Alcaligenes sp. AY238499
D2-8 (2)
B2-2
F4-19* / Massilia plicata AY966000 (2)
D2-11
D2-21 / Burkholderia sp. AB269813 (2)
D2-27
Comamonadaceae sp. AJ556799
D2-10 (2)
Glacier bacterium AY315181
D2-18 (9)
β-PROTEOBACTERIA
B2-16 (3)
Pseudoalteromonas sp. EF375572
Methylophaga sp. DQ486478
F4-27 (2)
B2-11 (2)?
Pseudoxanthomonas sp. AM403237
B2-27 (2)
Pseudomonas sp. EF523609
F4-35 (2)
Gamma Proteob. (SAR92) EU182722
B2-13
Microbulbifer sp. AB107975
F8-6 / Marine Gamma Prot. AY386345 (2)
F8-3
uncult. SAR86 DQ810396
F8-2 (2)
B2-1
Cellvibrio vulgaris AF448513
D2-2
Acinetobacter sp. EF514909
Vesicomya gigas AF035726
F4-39 (2)
γ-PROTEOBACTERIA
B2-32
Alpha proteob. SK200b-6 AB231963
B2-4
F4-5 / Roseobacter sp. ANT909 AY167254 (8)
F8-26
Iodiodide-oxidizing bacterium AB159210
F8-17 (2)
F4-14
F8-28
F4-8 / Pelagibacter ubique AF510191 (10)
α-PROTEOBACTERIA
B2-24
Aerococcus viridans AY707781
FIRMICUTES
F4-13 (2)
Micromonas sp. AY702163
Ochromonas danica chloropl. X531183
D2-9 (2)
F4-37
Apedinella radians chloropl. AY929296
F8-7
Dinophysis mitra chloropl. AB199887
Ankylochrysis lutea plast. AY702146
F4-4 (5)
CHLOROPLAST / PLASTID
F8-9b* / Microbacteriaceae bact. DQ490446 (3)
F4-16 (2)
D2-5 / Glacier bact. AY315169 (4)
B2-9
Sporichthya brevicatena AB006164
D2-1 (3)
B2-7
Microthrix parvicella X93044
ACTINO-BACTERIA
B2-17
Acidobacter bact. AB245337
ACIDOBACTERIA
B2-18
Pedobacter aurantiacus DQ235228
B2-22
Cytophagales sp. D84607
F4-40 (4)
F4-22
Cf. Cytophaga sp. AF530158
F4-21 (2)
Flavobacterium sp. DQ514307
F8-31
F8-35
B2-20
Marine psychrophile MPU85882
F4-15 (2)
Psychroserpens sp. DQ073103
F4-10 / Polaribacter irgensii AY167325 (2)
Polaribacter irgensii AY771712
F4-12 (3)
CFB
0.05

DISCUSSION

High taxonomic diversity, e.g. up to 134 taxa, has been reported from microscopic surveys of the pelagic community from Kongsfjorden (Keck *et al.* 2001; Hop *et al.* 2002). Keck *et al.* (2001) calculated taxonomic diversity indices of 1.4 at the outer Kongsfjorden and 3.4 near the glaciers. Although we cannot directly compare diversity indices obtained via different techniques and given reported drawbacks of DGGE analysis (Piquet *et al.* 2008). The number of bands detected in the DGGE patterns and the number of OTUs with at least 97% identity detected by sequencing (41 and 54 from the 18S rRNA and 16S rRNA, respectively) yielded an average diversity of 2.5 by molecular approach. This is comparable to previous findings for the Kongsfjorden micro-eukaryotic community and confirms the relatively high diversity of the Kongs-Krossfjorden system.

The observed eukaryotic community composition fits the typical sub-polar post-spring bloom as reviewed by Hop *et al.* (2002). It appeared that the spring bloom had passed, which normally occurs after sea-ice melting or, if sea-ice is absent, as soon as light becomes non-limiting. Earlier surveys on the Kongsfjorden phytoplankton reported spring blooms of diatoms, *Phaeocystis pouchetii* or *Dinobryon* sp. (*Chrysophyceae*) occurring from April until mid June (Eilertsen *et al.* 1989; Keck *et al.* 2001; Hop *et al.* 2002; Hop *et al.* 2006). *Bacillariophyceae* (diatoms) and *Haptophyceae* (including *Phaeocystis sp.*) were not detected in our sequences, although these are generally expected to be present in the Kongsfjorden (Hasle and Heimdal 1998; Keck *et al.* 2001). In 2005, winter sea ice had already dissapered early in the year resulting in an early onset and fall of a diatom bloom which might have led to the absence of these species in our surface water samples. Alternatively, our data can not exclude that typical marine phytoplankton species might have avoided the low-salinity surface layer and remained below the pycnocline.

Remarkably the micro-eukaryotic community fingerprints generated for the mid-Kongsfjorden location over a month period were very constant. This suggests that only a few micro-eukaryotic species were capable to cope with reduced salinities and high solar irradiation, and dominated throughout the sampling period. While the bacterial community fingerprints revealed variations over time that coincided with observed shifts in sediment load. Although we did not identify the bands observed in the DGGE, comparison of the microbial community fingerprint with the measured environmental variables (Table 5.1) suggests that the shift observed in weeks 4 and 5 might have been induced by changes in average wind direction, favouring the outflow of Bayelva sediment-rich water towards the sampling location TS. The observed increase in numbers of bands might have resulted from the inflow of bacteria attached to sediment particles, also providing an explanation for the appearance of bands in the larger size fractions in weeks 4 and 5.

The community fingerprints of eukaryotes and bacteria revealed by DGGE analysis clearly differed between the two fjords whereas the communities measured at different points within the same fjords remained relatively stable (Fig. 5.3). Although both fjords share a mouth to the open sea and are both under the influence of glacial meltwater, the presence of a bottom ridge at the outer part of the Krossfjorden (Fig. 5.1) may limit the inflow of WSC water and increase

the residence time of the water body as compared to the Kongsfjorden. The latter has an unlimited inflow of shelf water from the WSC and a faster turnover time (Svendsen *et al.* 2002). Moreover, the Krossfjorden is characterized by clear water with a deep-blue coloration, while the Kongsfjorden surface water has a brown-reddish coloration resulting from a much stronger sediment input. Differences in water body and sediment load were most likely responsible for the observed differences in microbial communities between both fjords.

Kongsfjorden phytoplankton mostly consists of Atlantic or cosmopolitan species, while only 31% are typical Arctic or boreal-Arctic species (Hop *et al.* 2006). At the same time, high inter-annual variability exists. This was shown by zooplankton community studies (Hop *et al.* 2002; Knasniewski *et al.* 2003) and coccolithophorid analyses, indicator species of occasional Atlantic water inflow events (Hasle and Heimdal 1998). Microscopy analysis of our Lugol preserved samples confirmed the relative dominance of dinoflagellates and tintinnids (*Ciliophora*) in our samples. Tintinnids have been reported to totally graze down blooms of, for example, *Phaeocystis* sp. and the red-tide dinoflagellate *Gyrodinium mikimitoi* (Admiraal and Venekamp 1986; Weisse and Scheffel-Möser 1990; Nakamura *et al.* 1995). Their presence in our samples suggests a high grazing pressure during the sampling period.

Sequencing data showed that the dinoflagellates were the most diverse group. Species related to *Gyrodinium rubrum*, *Gyrodinium spirale*, *Gymnodinium beii*, *Gymnodinium catenatum*, and a naked dinoflagellate related to *Scripsiella* sp. formed the majority of dinoflagellate OTUs. This strong dinoflagellate dominance substantiates their proposed central role in the post-spring microbial food-web as suggested by studies conducted in the Antarctic (Díez *et al.* 2004; Gast *et al.* 2006). Interestingly, OTU BF2-13 was closely related to the *Dinophyceae* sp. strain RS-24 isolated from the Antarctic Ross Sea (Gast *et al.* 2006); with 99% similarity over a 500 bp stretch this strain might have a bi-polar distribution. Our findings for the Kongsfjorden micro-eukaryotic community composition strongly contrast with data from Lovejoy *et al.* (2002), who conducted a thorough taxonomic survey of another Arctic marine system: the North Water Polynya. They showed that diatoms dominated the community at all locations throughout the survey period (April to July), except for the West Greenland Current region, where flagellates were dominant. In contrast to the Kongsfjorden, the North Water Polynya is characterized by nutrient-rich waters, providing enough substrate for a prolonged diatom bloom. This underlines the limitations of comparisons between marine Arctic systems, sharing a polar positioning, but governed by different water masses each with distinct nutritional properties.

As expected, our study reveals clear differences between the freshwater and marine eukaryotic communities. Among the main phyla detected: *Stramenopiles* (*Chrysophyceae* and *Synurophyceae*), *Fungi* and unclassified *Telonema* sp. related eukaryotes were confined to the freshwater habitats; only *Spumella*-like flagellate and *Epipyxis pulchra*, members of the *Stramenopiles*, are typical freshwater eukaryotes. All other phyla were detected in both marine and freshwater samples. In general, clones of marine samples formed distinct OTUs from the freshwater clones, however a few exceptions were found: the *Gyrodinium rubrum* related OTU F5-2-3, the *Gymnodinium catenatum* related OTU F5-2-17 and the cercozoan *Cryothecomonas longipes* related OTU BF-2-8, included clones from the freshwater and marine habitat. The

closest sequence matches revealed by BLAST queries were all from marine organisms. This suggests that the *Gyrodinium* sp., *Gymnodinium* sp., and *Cryothecomonas* sp. genus have closely related members found in marine and freshwater habitats.

In the Kongs-Krossfjorden system, α-*Proteobacteria* including the SAR11 cluster, dominated the clone library. Moreover members of the *γ-Proteobacteria* and *Cytophaga-Flavobacteria-Bacteroides* (CFB) formed a large proportion of the clone library. This is in agreement with other diversity studies conducted on marine polar bacteria (Bowman *et al.* 2000; Bano and Hollibaugh 2002; Morris *et al.* 2002). We also recovered sequences related to the β-*Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Acidobacteria*.

β-*Proteobacteria* generally constitute the dominant fraction of the community in freshwater systems, while they generally form a minority in marine systems (Methé *et al.* 1998; Glöckner *et al.* 1999). Here the F4-7 clone was most related to uncultivated β-*Proteobacteria* sequences recovered from marine systems. While the F8-4 clone matched β-*Proteobacteria* sequences previously recovered from Arctic (Bench Glacier, Alaska) and West Antarctic (Kamb ice stream) subglacial systems (Skidmore *et al.* 2005; Lanoil *et al.* 2009). The closest cultivated relatives of these environmental clones (BG.b9,- h6, and KIS77) are neutrophilic iron oxidizers belonging to the *Gallionella* genus, suggesting that these clones represent bacteria capable of iron oxidation. The Kongsbreen glacier meltwater is a major source of red-sediment enriched in iron oxide (S. Troelstra, *pers. comm.*). Overall, it is likely that F8-4 originated Kongsbreen glacier meltwater, moreover it also suggests the presence of iron-oxidizing bacteria within the Kongsbreen glacial system. Inflow of β-*Proteobacteria* from freshwater sources has already been reported for temperate marine sites (Crump *et al.* 1999; Glöckner *et al.* 1999). While in the Arctic, Garneau *et al*. (2005) studied the bacterial community composition at a coastal site influenced by the Mackenzie River and reported a significant decrease in β-*Proteobacteria* abundance in relation to increasing salinity. So far only a few studies have reported the presence of β-*Proteobacteria* in polar marine habitats. Sequences related to the *Nitrosomas* and *Nitrosospira* (ammonia-oxidizing β-*Proteobacteria*) were described for the Arctic Ocean (Hollibaugh *et al.* 2002), sequences related to the OM43 clade were recovered from the Beaufort Sea (Arctic) and the Kerguelen convergent in the Southern Ocean (Galand *et al.* 2008; West *et al.* 2008). Furthermore sequences related to the *Neisseriales* (marine bacteria group I) and *Burkholderiales* were recovered from a coastal Antarctic site in the Ross Sea (Gentile *et al.* 2006). Other β-*Proteobacteria* were mainly recovered from Arctic and Antarctic freshwater habitats (Methé *et al.* 1998; Pearce *et al.* 2005) and from freshwater melt pools of Arctic pack ice (Brinkmeyer *et al.* 2004). The presence of "non-marine" β-*Proteobacteria* in our marine samples indicates that glacial meltwater input is probably responsible for a continuous inflow of typical freshwater species. The detection F4-19, most related to β-*Proteobacteria* clones to sequences previously recovered from freshwater systems, from the outer Krossfjorden sampling location (F4) reveals the dispersal potential provided by meltwater outflow.

In the studied system, both turnover time of the water body and sediment load appear to be the major determinants of microbial community composition. There also appeared to be an input of freshwater species, especially bacteria, into the marine system via meltwater. Both an increased freshwater input into the marine system resulting from melting glaciers and snow/ice fields plus an increase in sediment runoff from the land are likely to occur as a consequence of the currently observed global temperature rise. Our data show that both parameters are important determinants for microbial diversity and thus suggest that global warming may dramatically affect ecosystem stability. This first molecular microbial diversity study of this unique ecosystem shows that further analysis is highly recommended and should consist of a multidisciplinary approach that studies the effect of increased meltwater and sediment influx not only on microbial population diversity at the surface of the water column but also of the geochemistry and populations of higher trophic levels of both surface and deeper waters.

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